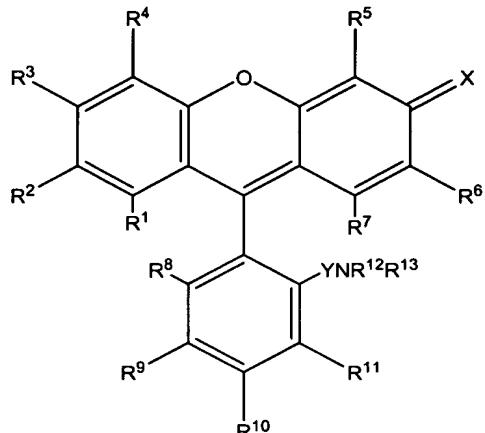


**WHAT IS CLAIMED IS:**

1. A xanthene dye having the formula:



2

### 3 in which

4           R<sup>1</sup>, R<sup>2</sup>, R<sup>4</sup>, R<sup>5</sup>, R<sup>6</sup>, R<sup>7</sup>, R<sup>8</sup>, R<sup>9</sup>, R<sup>10</sup> and R<sup>11</sup> are independently selected from substituted  
 5           or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or  
 6           unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or  
 7           unsubstituted heterocycloalkyl, halogen, H, NO<sub>2</sub>, CN and C(Z<sup>1</sup>)R<sup>14</sup>, NR<sup>15</sup>R<sup>16</sup>  
 8           and Z<sup>2</sup>R<sup>16</sup>:

$R^3$  is selected from  $Z^2R^{16}$  and  $NR^{15}R^{16}$

10 wherein

11 Z<sup>1</sup> is a member selected from O, S and NH:

12  $Z^2$  is a member selected from  $\Omega$  and  $S$ :

13 R<sup>15</sup> is a member selected from H, substituted or unsubstituted alkyl, and  
14 substituted or unsubstituted heteroalkyl;

15 R<sup>16</sup> is selected from H, substituted or unsubstituted alkyl, substituted or  
16 unsubstituted heteroalkyl, C(Z<sup>3</sup>)R<sup>17</sup>, and a nitrogen-containing reactive  
17 group comprising R<sup>15</sup> and R<sup>16</sup>, together with the nitrogen to which they  
18 are attached, wherein said reactive group is a member selected from  
19 -NHNH<sub>2</sub>, -N=C=S and -N=C=O

20 wherein

21  $Z^3$  is a member selected from O, S and NH;

22 R<sup>17</sup> is a member selected from substituted or unsubstituted alkyl,  
23 substituted or unsubstituted heteroalkyl, OR<sup>18</sup>, and NR<sup>19</sup>R

24 wherein

$R^{18}$  is a member selected from H, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl and  $C(O)R^{21}$

wherein

$R^{21}$  is substituted or unsubstituted alkyl or substituted or unsubstituted heteroalkyl;

$R^{19}$  and  $R^{20}$  are members independently selected from H, substituted or unsubstituted alkyl and substituted or unsubstituted heteroalkyl

Y is a member selected from C(O) and S(O)<sub>2</sub>;

X is a member selected from (NR<sup>22</sup>R<sup>23</sup>) and (O)

wherein

$R^{22}$  and  $R^{23}$  are members independently selected from H, substituted or unsubstituted alkyl and substituted or unsubstituted heteroalkyl; and  $R^{12}$  and  $R^{13}$  are members independently selected from substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl and substituted or unsubstituted heteroaryl, with the proviso that at least one of  $R^{12}$  or  $R^{13}$  comprises a member selected from a bond to a carrier molecule, a bond to a linker bound to a carrier molecule, a bond to a solid support, a bond to a linker attached to a solid support, a bond to a fluorescence quencher, a bond to a linker to a fluorescence quencher and an oxygen-containing reactive group, and further with the proviso that when  $R^{12}$  and  $R^{13}$ , together with the nitrogen to which they are attached form a piperazine ring said oxygen-containing reactive group is a phosphoramidite and said bond to a carrier molecule is other than a bond to a peptide.

1                           2.       The xanthene dye according to claim 1, wherein  $R^3$  is  $R^{15}R^{16}N$ ; and X  
2    is  $NR^{23}R^{24}$ , wherein  $R^{15}$ ,  $R^{16}$ ,  $R^{23}$  and  $R^{24}$  are members independently selected from H,  
3    substituted or unsubstituted alkyl and substituted or unsubstituted heteroalkyl.

1                           3.       The xanthene dye according to claim 1, wherein at least one of  $R^8$ ,  $R^9$ ,  
2     $R^{10}$  and  $R^{11}$  is a halogen.

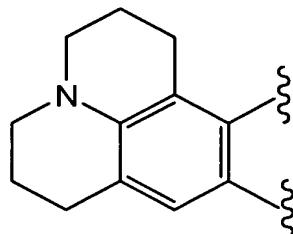
1                   4. The xanthene dye according to claim 1, wherein R<sup>9</sup> and R<sup>10</sup> are  
2   halogen.

1                   5. The xanthene dye according to claim 3, wherein R<sup>3</sup> is OR<sup>16</sup>; and X is  
2   O.

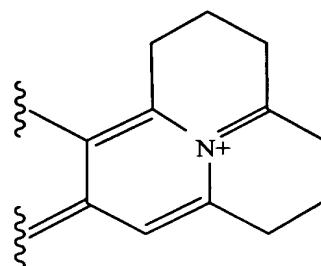
1                   6. The xanthene dye according to claim 5, wherein R<sup>2</sup> and R<sup>6</sup> are halogen.

1                   7. The xanthene dye according to claim 5, wherein R<sup>2</sup> and R<sup>6</sup> are  
2   independently selected from substituted or unsubstituted alkyl and substituted or  
3   unsubstituted heteroalkyl.

1                   8. The xanthene dye according to claim 1, wherein R<sup>3</sup> is NR<sup>15</sup>R<sup>16</sup> and R<sup>2</sup>,  
2   R<sup>4</sup> and R<sup>15</sup> and R<sup>16</sup>, together with the nitrogen atom to which they are bound, are fused with  
3   the phenyl moiety to which NR<sup>15</sup>R<sup>16</sup>, R<sup>2</sup> and R<sup>4</sup> are bound, forming a substituted or  
4   unsubstituted ring system having formula:



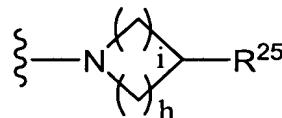
1                   9. The xanthene dye according to claim 1, wherein X is NR<sup>22</sup>R<sup>23</sup> and R<sup>5</sup>,  
2   R<sup>6</sup> and R<sup>22</sup> and R<sup>23</sup>, together with the nitrogen atom to which they are bound, are fused with  
3   the unsaturated 6-member ring to which NR<sup>22</sup>R<sup>23</sup>, R<sup>5</sup> and R<sup>6</sup> are bound, forming a substituted  
4   or unsubstituted ring system having the formula:



1                   **10.**    The xanthene dye according to claim 1, wherein said oxygen-  
2 containing reactive functional group is a member selected from hydroxyl and activated  
3 derivatives thereof, phosphoramidite, and carboxylic acid and activated derivatives thereof.

1                   **11.**    The xanthene dye according to claim 1, wherein  $R^{12}$  and  $R^{13}$ , together  
2 with the nitrogen to which they are bound are joined to form a ring system.

1                   **12.**    The xanthene dye according to claim 11, wherein  $NR^{12}R^{13}$  has the  
2 formula:

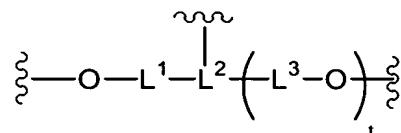


3                   wherein

5                   h and i are members independently selected from integers such that the sum (h + i) is  
6                   from 4-8; and

7                    $R^{25}$  is a reactive functional group.

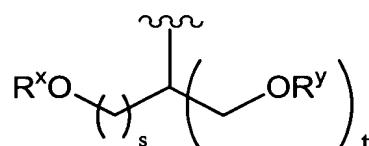
1                   **13.**    The xanthene dye according to claim 1, wherein  $R^{12}$  comprises a  
2 moiety having the formula:



3                   wherein

5                    $L^1$ ,  $L^2$  and  $L^3$  are members independently selected from substituted or  
6                   unsubstituted alkyl and substituted or unsubstituted heteroalkyl; and  
7                   t is 0 or 1.

1                   **14.**    The xanthene dye according to claim 13, said moiety having the  
2 formula:



3                   wherein

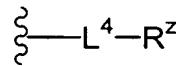
5                   R<sup>x</sup> and R<sup>y</sup> are members independently selected from H, substituted or  
6                   unsubstituted alkyl, substituted or unsubstituted heteroalkyl, a  
7                   hydroxyl-protecting group, a phosphate moiety, a phosphodiester  
8                   moiety, a phosphorus-containing internucleotide bridge of a nucleic  
9                   acid, a solid support, a carrier molecule and —OP(O)(OR<sup>o</sup>)(N(R<sup>p</sup>R<sup>q</sup>))<sub>2</sub>  
10                  wherein

11                  R<sup>o</sup>, R<sup>p</sup> and R<sup>q</sup> are members independently selected from H, substituted  
12                  or unsubstituted C<sub>1</sub>-C<sub>6</sub> alkyl and substituted or unsubstituted  
13                  C<sub>1</sub>-C<sub>6</sub> heteroalkyl; and

14                  s is an integer from 1 to 20.

1                  **15.**       The xanthene dye according to claim 14, wherein R<sup>o</sup> is CH<sub>2</sub>CH<sub>2</sub>CN.

1                  **16.**       The xanthene dye according to claim 14, wherein at least one of R<sup>x</sup> and  
2                  R<sup>y</sup> comprises a moiety having the formula:

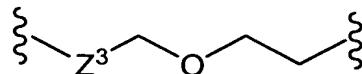


4                  wherein

5                  L<sup>4</sup> is a member selected from a bond, substituted or unsubstituted alkyl and  
6                  substituted or unsubstituted heteroalkyl; and

7                  R<sup>z</sup> is a member selected from a reactive functional group, solid support, a  
8                  nucleic acid, a saccharide and a peptide.

1                  **17.**       The xanthene dye according to claim 16, wherein L<sup>4</sup> comprises a  
2                  moiety having the formula:



4                  wherein Z<sup>3</sup> is a member selected from CH<sub>2</sub> and C=O.

1                  **18.**       The xanthene dye according to claim 1, wherein said carrier molecule  
2                  further comprises a quencher moiety.

1                  **19.**       The xanthene dye according to claim 18, wherein said xanthene dye  
2                  and said quencher comprise a donor-acceptor energy transfer pair.

1                   **20.**    The xanthene dye according to claim 18, wherein said quencher has  
2 substantially no native fluorescence.

1                   **21.**    The xanthene dye according to claim 20, wherein said quencher  
2 comprises at least three residues selected from aryl, substituted aryl, heteroaryl, substituted  
3 heteroaryl and combinations thereof, wherein at least two of said residues are covalently  
4 linked via an exocyclic diazo bond.

1                   **22.**    The xanthene dye according to claim 1, wherein said xanthene dye is  
2 attached to a nucleic acid at a position which is a member selected from the 3'-terminus, the  
3 5'-terminus, a nucleobase, and a phosphorus-containing internucleotide bridge of said nucleic  
4 acid.

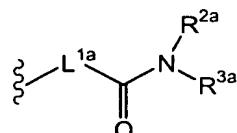
1                   **23.**    The xanthene dye according to claim 18, wherein said nucleic acid is a  
2 probe which is a member selected from molecular beacons, scorpion probes, sunrise probes,  
3 conformationally assisted probes and TaqMan™ probes.

1                   **24.**    The xanthene dye according to claim 1, wherein said carrier molecule  
2 is a peptide comprising a cleavage recognition site for an enzyme.

1                   **25.**    The xanthene dye according to claim 24, wherein said peptide  
2 comprises a cleavage recognition site for a protease.

1                   **26.**    The xanthene dye according to claim 24, wherein said cleavage  
2 recognition site is for an enzyme selected from trypsin, enterokinase, HIV-1 protease,  
3 prohormone convertase, interleukin-1b-converting enzyme, adenovirus endopeptidase,  
4 cytomegalovirus assemblin, leishmanolysin,  $\beta$ -secretase for amyloid precursor protein,  
5 thrombin, renin, angiotensin-converting enzyme, cathepsin-D and a kininogenase.

1                   **27.**    The xanthene dye according to claim 1, in which R<sup>12</sup> has the formula:



2                   wherein

4                   L<sup>1a</sup> is a member selected from substituted or unsubstituted alkyl, and  
5                   substituted or unsubstituted heteroalkyl groups; and

6                   R<sup>2a</sup> and R<sup>3a</sup> are members independently selected from H, substituted or  
7                   unsubstituted alkyl, and substituted or unsubstituted heteroalkyl, and  
8                   R<sup>2</sup> and R<sup>3</sup>, together with the nitrogen to which they are attached, are  
9                   optionally joined to form a ring which is a member selected from  
10                  substituted or unsubstituted C<sub>5</sub>-C<sub>7</sub> cycloalkyl and substituted or  
11                  unsubstituted 5-7-membered heterocycloalkyl.

1                   28. The xanthene dye according to claim 27, in which L<sup>1a</sup> does not  
2                  comprise a member selected from a carboxylic acid and a carboxylic acid ester.

1                   29. A method for determining whether a sample contains an enzyme, said  
2                  method comprising:

- 3                   (a) contacting said sample with a peptide construct comprising
  - 4                   i) a xanthene dye according to claim 1;
  - 5                   ii) a quencher; and
  - 6                   iii) a cleavage recognition site for said enzyme,  
7                   wherein said peptide is in a conformation allowing donor-acceptor energy  
8                   transfer between said fluorophore and said quencher when said  
9                   fluorophore is excited;
- 10                  (b) exciting said xanthene dye; and
- 11                  (c) determining a fluorescence property of said sample, wherein the presence of said  
12                  enzyme in said sample results in a change in said fluorescence property.

1                   30. A method for determining whether a compound alters an activity of an  
2                  enzyme, said method comprising:

- 3                   (a) contacting a sample comprising said enzyme and said compound with a peptide  
4                   construct comprising
  - 5                   i) a xanthene dye according to claim 1;
  - 6                   ii) a quencher; and
  - 7                   iii) a cleavage recognition site for said enzyme,  
8                   wherein said peptide is in a conformation allowing donor-acceptor energy  
9                   transfer between said xanthene dye and said quencher when said xanthene  
10                  dye is excited;
- 11                  (b) exciting said xanthene dye; and

12 (c) determining a fluorescence property of said sample, wherein said activity of said  
13 enzyme in said sample results in a change in said fluorescence property.

1 31. A method for detecting a nucleic acid target sequence, said method  
2 comprising:

3 (a) contacting said target sequence with a detector oligonucleotide comprising a  
4 target binding sequence, said detector oligonucleotide having linked thereto,  
5 i) a xanthene dye according to claim 1; and  
6 ii) a quencher,  
7 wherein said detector nucleic acid is in a conformation allowing donor-  
8 acceptor energy transfer between said xanthene dye and said quencher  
9 when said xanthene dye is excited;

10 (b) hybridizing said target binding sequence to said single-stranded target sequence,  
11 thereby altering said conformation of said detector oligonucleotide, causing a  
12 change in a fluorescence parameter; and  
13 (c) detecting said change in said fluorescence parameter, thereby detecting said  
14 nucleic acid target sequence.

1 32. The method according to claim 31, wherein said complementary strand  
2 is synthesized in a target amplification reaction.

1 33. The method according to claim 31, wherein said complementary strand  
2 is synthesized by extension of the target sequence using said detector oligonucleotide as a  
3 template.

1 34. The method according to claim 31, wherein said fluorescence  
2 parameter is detected in -real-time.

1 35. A method for detecting amplification of a target sequence comprising,  
2 in an amplification reaction:

3 (a) hybridizing to said target sequence a detector oligonucleotide comprising a single-  
4 stranded target binding sequence and an intramolecularly associated secondary  
5 structure 5' to said target binding sequence, wherein at least a portion of said  
6 detector sequence is a single stranded tail which is available for hybridization to  
7 said target sequence, said detector oligonucleotide having linked thereto,  
8 i) a xanthene dye according to claim 1; and

ii) a quencher,  
wherein said detector nucleic acid is in a conformation allowing donor-acceptor energy transfer between said xanthene dye and said quencher when said xanthene dye is excited;  
tending said hybridized detector oligonucleotide on said target sequence with a polymerase to produce a detector oligonucleotide extension product and separating said detector oligonucleotide extension product from said target sequence;  
bridizing a primer to said detector oligonucleotide extension product and ending the primer with said polymerase, thereby linearizing said ramolecularly associated secondary structure and producing a change in a fluorescence parameter; and  
etecting said change in said fluorescence parameter, thereby detecting said target sequence.

36. The method according to claim 35, wherein said target sequence is amplified by a method selected from Strand Displacement Amplification, Polymerase Chain reaction, Self Sustained Sequence Replication, Transcription Mediated Amplification, and Nucleic Acid Sequence Based Amplification.

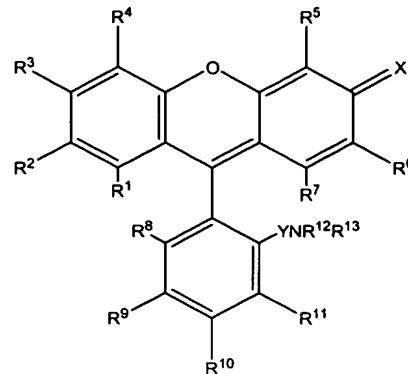
37. The method according to claim 35, wherein said secondary structure further comprises a partially or entirely single-stranded restriction endonuclease site.

**38.** The method according to claim 35, wherein a change in fluorescence intensity is detected.

**39.** The method according to claim 38, wherein said change in fluorescence intensity is detected in real-time.

40. The method according to claim 35, wherein said intramolecularly associated secondary structure comprises a portion of said target binding sequence.

41. A method of preparing a conjugate between a nucleic acid and a xanthene dye having the formula:



3

4 in which

5  $R^1, R^2, R^4, R^5, R^6, R^7, R^8, R^9, R^{10}$  and  $R^{11}$  are independently selected from substituted  
6 or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or  
7 unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or  
8 unsubstituted heterocycloalkyl, halogen, H,  $NO_2$ , CN and  $C(Z^1)R^{14}$ ,  $NR^{15}R^{16}$   
9 and  $Z^2R^{16}$ ;

10  $R^3$  is selected from  $Z^2R^{16}$  and  $NR^{15}R^{16}$

11 wherein

12  $Z^1$  is a member selected from O, S and NH;

13  $Z^2$  is a member selected from O and S;

14  $R^{15}$  is a member selected from H, substituted or unsubstituted alkyl, and  
15 substituted or unsubstituted heteroalkyl;

16  $R^{16}$  is selected from H, substituted or unsubstituted alkyl, substituted or  
17 unsubstituted heteroalkyl,  $C(Z^3)R^{17}$ , and a nitrogen-containing reactive  
18 group comprising  $R^{15}$  and  $R^{16}$ , together with the nitrogen to which they  
19 are attached, wherein said reactive group is a member selected from  
20  $-NHNH_2$ ,  $-N=C=S$  and  $-N=C=O$

21 wherein

22  $Z^3$  is a member selected from O, S and NH;

23  $R^{17}$  is a member selected from substituted or unsubstituted alkyl,  
24 substituted or unsubstituted heteroalkyl,  $OR^{18}$ , and  $NR^{19}R^{20}$

25 wherein

26  $R^{18}$  is a member selected from H, substituted or unsubstituted  
27 alkyl, substituted or unsubstituted heteroalkyl,  
28 substituted or unsubstituted aryl, substituted or  
29 unsubstituted heteroaryl and  $C(O)R^{21}$

30 wherein  
31  $R^{21}$  is substituted or unsubstituted alkyl or substituted or  
32 unsubstituted heteroalkyl;  
33  $R^{19}$  and  $R^{20}$  are members independently selected from H,  
34 substituted or unsubstituted alkyl and substituted or  
35 unsubstituted heteroalkyl  
36 Y is a member selected from C(O) and S(O)<sub>2</sub>;  
37 X is a member selected from (NR<sup>22</sup>R<sup>23</sup>) and (O)  
38 wherein  
39  $R^{22}$  and  $R^{23}$  are members independently selected from H, substituted or  
40 unsubstituted alkyl and substituted or unsubstituted heteroalkyl; and  
41  $R^{12}$  and  $R^{13}$  are members independently selected from substituted or  
42 unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted  
43 heterocycloalkyl, substituted or unsubstituted aryl and substituted or unsubstituted heteroaryl,  
44 with the proviso that at least one of  $R^{12}$  or  $R^{13}$  comprises said nucleic acid,  
45 said method comprising:  
46 (a) contacting a precursor of said conjugate comprising nucleic acid protecting  
47 groups with a mixture of amine and alcohol, thereby removing said protecting groups.